On Being Human: Life in the Microbiome

When leaders of the Human Genome Project promised new insight into what it means to be human, they did not have microorganisms in mind. With massive advances in tools to rapidly sequence and assemble billions of DNA base pairs—which have seen sequencing costs drop by orders of magnitude—clinical researchers were understandably focused on decoding human genes. But the beauty of scientific inquiry is that a journey toward one destination often leads to unanticipated treasure troves. The new sequencing technologies have quickly given scientists an unprecedented view not only of the human genome, but of the ecosystems of microbial life forms that share the human epithelium. CCR scientists are embracing the microbiome as a heretofore unrecognized and potentially important player in human health and disease.

Skin Deep in Microbes

"One of our approaches to understanding disease is to step back and determine what is considered healthy or normal," said Heidi Kong, M.D., M.H.Sc., Investigator in CCR's Dermatology Branch. Her interest in diseases of the skin, made its microbial landscape a natural research subject. Kong and her intramural collaborator, Julie Segre, Ph.D., Chief of the National Human Genome Research Institute's Translational and Functional Genomics Branch, have been working together for several years to map the microbial ecosystem of the skin.

The presence and diversity of microbes have been vastly underestimated in many contexts, primarily because these organisms have been so difficult to study. Typically, researchers have needed to grow a critical mass of a given bacterium or fungus in a dish. "Culturing techniques depend on certain conditions, so there are many microbes that can be difficult to isolate," explained Kong. "Genomic methodologies are somewhat less biased in their assessment of diversity."

(Photo: R. Baer)

Heidi Kong, M.D, M.H.Sc., collects skin samples with the assistance of Research Nurse Shelia Phang.

In one of their first papers, which appeared in *Science* in 2009, Kong, Segre, and their colleagues looked at bacterial diversity on multiple skin sites on the human body. All prokaryotes have distinct sequences of rRNA that comprises the 16S small-subunit of the ribosome. Part of the sequence is variable among species, which makes it suitable for bacterial identification; part is highly

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conserved which means it can be easily targeted for sequencing. By sequencing the 16S ribosomal RNA from 20 distinct skin sites, Kong and Segre found patterns of microbial species related to skin type: oily, dry, or moist.

"We showed that two skin sites a few inches apart might have quite different microbial communities," said Kong. This result not only spoke to the diversity of the skin microbiome, but the need for very careful sampling in ongoing studies. "Human microbial load on the skin is very low, as compared to stool samples, for example," said Kong. "We've had to optimize clinical protocols and methods in the laboratory to obtain robust results."

Kong and her team went on to examine bacterial communities in skin diseases such as atopic dermatitis, a common chronic inflammatory skin condition also known as eczema, that has been on the rise among children in the industrialized world. The disease has been associated with *Staphylococcus aureus* and is commonly treated with antibiotics and corticosteroids. By looking at patients over multiple time points, during and between flare-ups of the disease, they found that inflammation was associated with an increase of both *S. aureus* and *S. epidermis*, and a decrease in overall bacterial diversity.

More recently, the researchers have modified their techniques to study fungi as well as bacteria. In addition to targeting a different ribosomal RNA gene, they also had to adapt their laboratory handling protocols to capture these distinct species. "The body sites with much more fungal diversity were on the feet, which is strikingly different from bacterial distributions," said Kong. They also looked at patients with primary immunodeficiencies, who often suffer from recurrent skin infections and found patterns of bacteria and fungi that aren't found in healthy individuals. "It suggests that the defect in their immune systems allows their skin to be more permissive for fungi and bacteria," said Kong.

(Photo: R. Baer)

Giorgio Trinchieri, M.D., Romina Goldszmid, Ph.D., and Marco Cardone, Ph.D., in the lab

In October 2014, Kong, Segre, and their colleagues published a paper in *Nature* describing the results of shotgun metagenomics sequencing, a technique that allowed them to capture the entire genomes of microbes present on the skin. "Instead of only looking at one group of microbes, now we look at all microbes *in toto*. We get a better sense of how the bacteria relate to the fungi and viruses in one sample. And with the whole genome, we potentially obtain some insight into the functional capacity of these microbes."

Getting beyond correlations is a key challenge for microbiome researchers. "With sequencing data, you are inundated with the millions of sequences, so trying to tease apart what all these data mean is a challenge. The key point is that these studies are helpful for identifying associations that can be further studied in a more targeted fashion, including a host immunological perspective."

A Cancer Ecosystem

Giorgio Trinchieri, M.D., Program Director of CCR's Cancer and Inflammation Program and Chief of the Laboratory of Experimental Immunology, has long focused his research on the interplay between innate and adaptive immune systems, and their role in cancer progression and therapy. The innate immune system is the evolutionarily older, first line of defense against a variety of threats, with cells ready to mobilize at a moment's notice. Trinchieri sees innate immunity as an adaptation of signaling mechanisms that evolved as eukaryotes emerged.

"As soon as these multicellular organisms formed, they were living in a world that was full of microbial bacteria, viruses, fungi, and so on. Multicellular organisms are symbionts of host cells with commensal microorganisms and cannot live without these interactions. What we use today for natural immunity—and even adaptive immunity—are the mechanisms that originally allowed the components of the symbionts to

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communicate with each other, maintain equilibrium, and avoid pathogens," explained Trinchieri.

This point of view led Trinchieri to consider how differences in an individual's microbiome might affect cancer and its treatment. Trinchieri and Staff Scientist Romina Goldszmid, Ph.D., led their colleagues in a study, published in *Science* in 2013, of three xenograft tumor models, based on lymphoma, carcinoma, and melanoma cell lines chosen for their sensitivity to specific therapies (see "Interspecies Cooperation to Fight Cancer," *CCR connections* Vol. 8, No 1). To deplete their microbiomes, mice were either pre-treated with a cocktail of antibiotics or raised from birth in a germ-free environment. Then, once the tumors reached a certain size, the mice were challenged with the appropriate immunotherapy or a conventional platinum-based chemotherapy.

Regardless of the tumor type or treatment examined, mice depleted of their gut microbes responded less well to therapy than controls, as measured by reduction in tumor volume. Moreover, the number of myeloid-derived cells infiltrating the tumors was reduced in the absence of microbiota and was associated with reduced tumor necrosis factor (TNF) production by myeloid cells in the case of immunotherapy and reduced reactive oxygen species (ROS) production after chemotherapy. Administration of bacterial products could restore TNF production by tumor myeloid cells in animals depleted of their gut microbiota. Allowing microbial colonies to reestablish after antibiotic treatment, they further determined that bacterial composition—and not just abundance—was critical.

"One big question that emerges is how changes in the gut microbiome (and possibly in other anatomical barrier sites), affect the way the inflammatory cells inside the tumors respond to different types of therapy," said Trinchieri. "In germ-free animals, we were quite surprised that there were only very minor differences in the numbers and gene expression parameters of inflammatory cells. But, when you treat the tumors, the response is completely different. We want to understand how the gut signals to these cells and what happens at the genetic and epigenetic level to prime them differently."

In addition to further work in animal models, Trinchieri and his colleagues are beginning to investigate the human microbiome, for example, by taking advantage of blood and microbiota samples from existing protocols in which volunteers are treated with antibiotics. "But we also want to look at cancer patients—characterizing their microbiota and their responses to treatments—and look epidemiologically at patients treated with antibiotics for different reasons."

"When you have any type of disease, but especially cancer, you must consider that it is growing in a metaorganism, which plays a role in creating the microenvironment in which the tumors grow and regulates the immune response. Even tumor therapy is going to be affected."

Metabolic Reduction

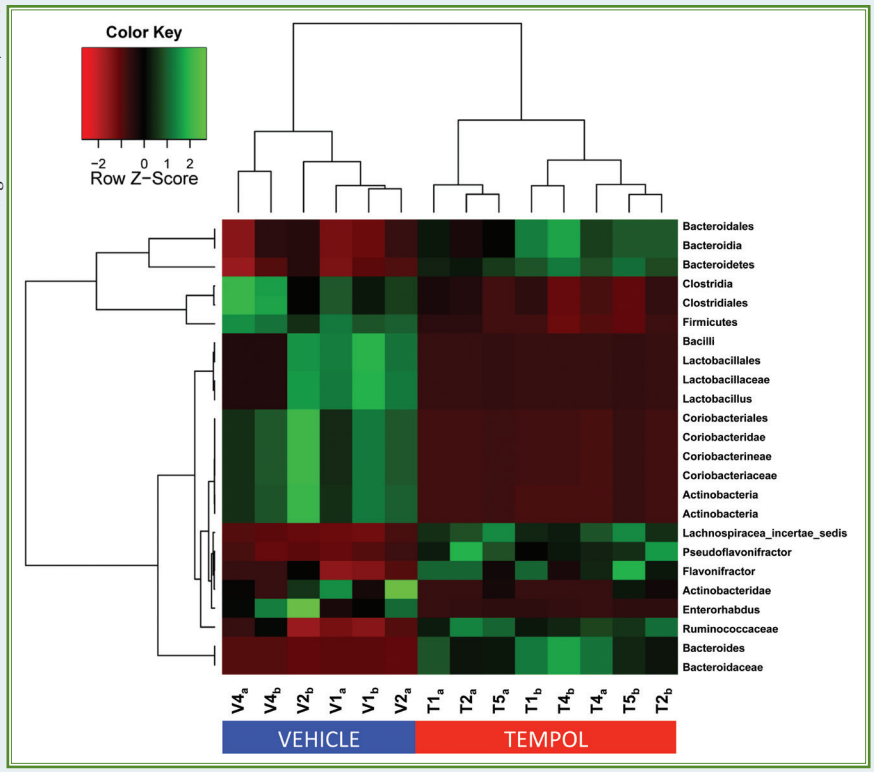
Frank Gonzalez, Ph.D., Chief of CCR's Laboratory of Metabolism, specializes in the use of liquid-chromatography mass spectrometry (LCMS) as a tool to explore the mechanisms of cancer progression and therapy. His colleague, James Mitchell, Ph.D., Chief of CCR's Radiation Biology Branch, asked him to take a look at the metabolomics profile of tempol, a nitroxide compound identified by Mitchell's group as a protectant against radiation damage and independently associated with obesity prevention and protection from insulin resistance in mice.

"We found altered levels of suspected gut microbe-generated metabolites indicating that tempol either changes the composition of the microbiome or alters the metabolic potential of gut bacteria," said Gonzalez.

Using 16S rRNA sequencing alongside metabolomics analysis, Gonzalez and his colleague Andrew Patterson, Ph.D., at Penn State University, found that tempol did indeed alter the gut microbiome, dramatically reducing both the genus *Lactobacillus* and levels of its enzyme bile salt hydrolase (BSH) in the feces. BSH enzymatically hydrolyzes conjugated bile acids produced in the liver; and the team found that the decreased BSH levels translated to an accumulation of one particular bile acid, tauro-β-muricholic acid, (T-β-MCA), an antagonist of the nuclear receptor farnesoid X receptor (FXR).

Modulation of the gut microbiota populations with either tempol or antibiotics decreases obesity in high-fat diet-fed mice and in genetically obese mice through inhibition of FXR signaling. It also improves insulin resistance (type 2 diabetes) and fatty liver disease, all of which are risk factors for cancer. Tempol would

(Figure: F. Gonzalez, CCR)



Gonzalez and colleagues found that tempol induces changes in the gut microbiome, including decreases in the family *Lactobacillaceae*. The heatmap of 16S rRNA gene sequence analysis depicts the microbial population found in caecum content after 5 days of treatment with tempol. The relative values for microbes are depicted by color: green colors indicate high values and red colors indicate low values.

not be a good therapeutic candidate because of the high doses required; the same beneficial results can be obtained by use of an FXR antagonist. Gonzalez and his colleagues have identified a derivative of T-β-MCA that exhibits the same efficacy toward metabolic diseases as modulation of the microbiota.

"We've finished the preclinical mouse studies filed a patent on this compound and its derivatives and are launching a study to treat diet-induced obese monkeys," said Gonzalez. "It's a promising compound because it is not absorbed into the bloodstream; it stays in the intestine and there's no indication of any intestinal toxicity in our studies. This is the first direct demonstration that metabolism by bacteria alters nuclear receptor signaling in the intestinal epithelium that effects obesity, diabetes, and fatty liver disease."

Being in the Minority

"Within CCR, there are many separate lines of investigation on the microbiome that were initiated for very different reasons, but that I think will merge very quickly," said Trinchieri.

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Trinchieri points to two new initiatives that should aid in investigations of the microbiome across CCR: a germ-free gnotobiotic facility at the NCI campus in Frederick and a CCR core facility for molecular and bioinformatics studies of the microbiome in Bethesda. Moreover, CCR and the National Institute of Allergy and Infectious Diseases intramural programs are actively engaged in efforts to provide integrated support as scientists with diverse research interests consider how to assess the impact of the microbiome on their findings. As recently reported by Michael Gottesman, M.D., NIH Deputy Director for Intramural Research, in *The NIH Catalyst*, research on the human microbiome and drug resistance will be included in "The Future of IRP" document as one of the areas of scientific opportunity in which the NIH Intramural Program is best poised to succeed.

"When we look at an organism like a human being, we should not just focus on its own cells. The organism is really a symbiote, a metaorganism," said Trinchieri. "In terms of numbers, we are up to 90 percent microbial cells."

To learn more about Dr. Gonzalez's research, please visit his CCR website at https://ccr.cancer.gov/frank-j-gonzalez.

To learn more about Dr. Kong's research, please visit her CCR website at https://ccr.cancer.gov/heidi-h-kong.

To learn more about Dr. Trinchieri's research, please visit his CCR website at https://ccr.cancer.gov/giorgio-trinchieri.